low unitary conductance, a sub-conductance level and a long open/closed dwell time[1]. From the effect of non-electrolyte polymers on this unitary conductance we estimate a narrow pore with a diameter of ca. 2.2 Å[2]. This value is similar to that reported for the central pore in the structure of the PLN pentamer[3]. Hence the PLN pentamer, which is in equilibrium with the monomer[4], is the most likely channel forming structure. Moreover, recent data on selectivity show that the channel follows the II Eisenmann sequence (Rb>Cs>K>Na>Li). This pattern reveals that the ion selectivity is regulated by the radius of the binding site and not by the ionic radius. Our results combined with computational data suggest the presence of a selectivity filter in the pentamer PLN.

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Ion Channels and Disease II

3851-Pos Board B579
TRPM4 Genetic Variants in Patients with Congenital Atrio-Ventricular Block

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Background: Transient Receptor Potential Subfamily Melastatin Member 4 (TRPM4) is a non-selective cardiac cation channel. Mutations in its gene cause cardiac conduction disease. The mechanisms underlying TRPM4-dependent conduction disorders are not fully understood.
Aim: To investigate genetic variants of TRPM4 from congenital atrio-ventricular block (cAVB) patients.
Methods and Results: Ninety-five cAVB patients were screened for candidate genes. We found 6 genetic variants in TRPM4. In whole-cell patch-clamp configuration, TRPM4 displays two current phases after patch rupture: transient (tr) and plateau (pl). Two cAVB mutants, p.A432T and p.A432T;G582S, showed lower protein expression (29 ± 4% and 17 ± 7% n=4) and one, p.G582S, showed higher expression (177 ± 15% n=4) compared to WT. Interestingly, only the plateau phase matches the protein expression profile (p.A432T 241 ± 23pA/pF n=6, p.G582S 994 ± 118pA/pF n=6 vs. WT 580 ± 66pA/pF n=20). Lower incubation temperature (28°C for 24h), which compromises protein quality control, rescued low expressing mutants, both at protein (p.A432T 72 ± 8% and p.A432T;G582S 65 ± 7% n=3) and functional (p.A432T 600 ± 114pA/pF n=6 vs. WT 639 ± 116pA/pF n=4) levels. We studied whether ubiquitylation plays a role in this loss-of-expression. Despite being ubiquitylated, no link between ubiquitylation and TRPM4 expression could be observed.
Conclusions: TRPM4 mutations in cAVB patients cause loss- or gain-of-function. Analogous to the supernormal conduction phenomenon, we speculate that increase or decrease of its activity in the conduction system may hyperpolarize or depolarize the resting membrane potential; thus, reducing the availability of sodium channels and subsequently leading to conduction slowing and clinical phenotype.

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The Contribution of Store Operated and Store Independent Calcium Entry to Migration in a Model of Neuroendocrine Cancer

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Orai1, the pore-forming sub-unit of a plasma membrane resident Ca^{2+}-channel and STIM-1, a Ca^{2+}-sensor localized to the endoplasmic reticulum are known to be involved in the canonical Store-operated Ca^{2+}-entry (SOCE) pathway. In recent years, this pathway has been implicated in cell migration of some types of cancers. However, it is not clear how near-maximal store depletion, a signal that is thought to be necessary for SOCE activation and generally considered pathophysiological, may effectively contribute to cell migration. Alternatively, a distinct, less well-studied store-independent channel type containing Orai1 and Orai3 has been shown to mediate Ca^{2+}-entry following activation by arachidonic acid. The relative contribution of store-operated and store-independent Ca^{2+}-entry pathways in cancer cell migration has not been adequately interrogated. The present study investigated this question in a model gastro-enteropancreatic neuroendocrine tumor (GEPNET) cell line by utilizing pharmacological and gene-silencing methods in combination with live cell fluorescence imaging and standard migration assays. It was revealed that these Ca^{2+}-entry pathways could be independently activated and regulated in these cells. While, classical SOCE required participation of Orai-1 and STIM1, the arachidonate mediated Ca^{2+}-entry pathway required in addition Orai3. Having identified both SOCE and arachidonate mediated Ca^{2+}-entry in GEPNET cells, their relative roles in cell migration were assessed using modified Boyden chamber assays. By selectively modulating channel conductances using pharmacological agents or sh-RNA to knockdown protein expressions, it was revealed that the arachidonate mediated Ca^{2+}-entry is the dominant pathway that induced migration in this type of cancer. Taken together, these data address the complex interaction between distinct Ca^{2+}-entry pathways that may induce cell migration yet utilize a common pool of molecular machinery.

3853-Pos Board B581
Inhibition of Mitochondrial Na^+/Ca^{2+} Exchanger Prevents Stress-Induced Arrhythmia in the Isolated Guinea Pig Failing Heart

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The rate of sudden cardiac death (SCD) in patient with heart failure (HF) is about 9 times higher than that in the general population; however, the mechanisms of HF-associated SCD are poorly understood. We previously demonstrated that stress (isoproterenol chanllenge) induced a burst of reactive oxygen species (ROS) in association with delayed afterdepolarizations (DAD) in isolated failing cardiomyocytes, and inhibition of mitochondrial Na^+/Ca^{2+} exchanger (mNCE) prevented ROS production and DAD. Here, we tested the hypothesis that isoproterenol challenge induces arrhythmias in the failing heart and that the mNCE inhibitor, CGP-37157 (CGP), has anti-arrhythmic effect. The guinea pig HF model, ACi, was produced with aorta constriction combined with daily isoproterenol injection for 4 weeks. Perfused shami (sham with daily isoproterenol injection), ACi, and ACi+CGP hearts were subjected to the following electrocardiographic recording protocol: 10-minute baseline, 15-minute with 10 μM isoproterenol, and 20-minute after washout of isoproterenol (post-iso). In ACi+CGP group, 1 μM CGP was added to the buffer at the beginning of baseline recording. Arrhythmias were evaluated with heart rate variability (HRV) analysis. Isolated ACi hearts did not show significant arrhythmia at baseline. Surprisingly, isoproterenol challenge did not induce overt arrhythmias during the period of maximum LVPD in either group. However, HRV was dramatically increased during post-iso state in ACi heart. SDNN (standard deviation of the normal beat RR interval), RMSSD (root mean square of successive differences in RR), and non-linear parameters, SD1 and SD2 during post-iso was 42.08 ± 8.51, 65.60 ± 15.60, 46.418 ± 11.048, and 36.542 ± 6.167 in ACi, respectively, whereas they were 3.85 ± 1.39, 0.88 ± 0.29, 0.626 ± 0.207, 5.394 ± 1.968 in shami, respecitively, $p \leq 0.05$. CGP-37157 treatment prevented HRV increase in ACi heart during post-iso. Our results suggest that mNCE is an important therapeutic target for HF-associated ventricular arrhythmia and SCD.

3854-Pos Board B582
Identification and Functional Role of Calpain Cleavage Site in Na^+-Ca^{2+} Exchanger 1 (NCX1)

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Altered Ca^{2+} homeostasis is a key determinant of cardiac remodeling and contractility during chronic heart disease. Aberrant activation of calpain, a ubiquitous Ca^{2+}-dependent protease can contribute to loss of Ca^{2+} control in cardiomyocytes. Calpain cleaves NCX1, but the underlying significance of the direct cleavage of NCX1 by calpain remains to be determined.
By bioinformatics and mutational analysis we identified M369 as a putative calpain cleavage site residing within the α-catenin-like domain (CLD) in NCX1. Importantly, the cleavage of NCX1 at M369 corresponded to a proteolytic fragment of 75 kDa in left ventricular biopsies from aorta stenosis (AS) patients and in the failing left ventricle of rats following aortic banding (AB). Moreover, calpain binding in the CLD and Ca^{2+} -binding domain (CBD1) of